TOPIC 18 – Electrophysiology, rythmology and pacing – E

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0186

Genetic study of cardiac calcium channel complex Cav1.2 in patients with cardiac arrhythmias

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Objective: The purpose of this study was to perform genetic screening of *CACNA1C*, *CACNB2*, and *CACNA2D1* in patients with arrhythmias to identify novel causal mutations.

Methods and results: Mutational screening of *CACNA1C* (53 exons), *CACNB2* (13 exons), and *CACNA2D1* (40 exons) was performed by high resolution melting (HRM) and direct DNA sequencing. The studied population was composed by 49 patients : 29 with BrS and relatively short QTc (QT corrected for heart rate) ranging from 325 to 370ms, 7 with short QT syndrome (QTc inferior to 330ms), 6 with ERS and 12 with IVF. Eleven novel variants were identified, 10 in *CACNA1C* and one in *CACNB2*. Among these variants, two missense mutations, absent in 300 unrelated controls were found in the C-terminal domain of CACNA1C in conserved domains, one in a patient with ERS and the other in a patient with IVF. The molecular and cellular mechanisms by which these variants alter the function of the calcium channel Cav1.2 will be further elucidated after heterologous expression of the WT and mutants.

Conclusion and perspective: This study does not identify mutations in the 24 BrS patients but provides two novel *CACNA1C* variants, one associated with IVF, and the other for the first time in this subunit with ERS. Presence of mutations in Cav1.2 subunits in more than 10% of the symptomatic patients with IVF and ERS should be confirmed by additional studies.

0365

Should programmed ventricular stimulation be performed to all patients with arrhythmogenic right ventricular cardiomyopathy?

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Background: Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D) is one of the major causes of sudden death in the young adult population. Risk stratification of sudden death and ventricular arrhythmias in these patients is the main clinical challenge for timely preventive strategies including drug therapy and implantable cardioverter-defibrillator (ICD). The usefulness of programmed ventricular stimulation (PVS) for risk stratification remains debatable.

Objective: to assess the PVS usefulness depending of clinical presentation

Methods and results: We enrolled 111 patients with probable or definite ARVD/C (mean age: 40.87 +/- 13years, 82 males (74%)) who had undergone PVS at time of diagnosis. Detailed clinical information regarding symptoms, ECG, 24-hour-holter monitoring, exercise stress testing, signal-averaged ECG, cardiac imaging and spontaneous ventricular tachycardia (VT) occurrence was obtained for each patient. 35/111 patients (32%) were first-degree relatives of proband patients, 42/111 (38%) were asymptomatic, 31/111 (28%) had spontaneous ventricular tachycardia (VT). Overall, 28/111 (25%) presented sustained VT inducibility at PVS. VT inducibility was remarkably lower in asymptomatic (n=2/45 (5%)) than in symptomatic patients (n=26/66 (40%); P=0.03). In the subgroup of asymptomatic first-degree relatives, PVS was negative in 99% of patients (n=26/27). The absence of symptoms was associated with a high negative predictive value of PVS (96%). PVS inducibility was statistically higher in patients who presented spontaneous VT at diagnosis (n=17/31 (54%) p<0.001) than in others (n=11/80 (13%)). The absence of spontaneous VT at diagnosis was associated with a 83% negative predictive value for PVS.

Conclusion: PVS is almost always negative in asymptomatic patients, especially in first-degree relatives and thus is probably not clinically relevant in this sub-group to assess risk stratification.

0287

Acylcarnitine: A link between oxidative metabolism and excitation contraction coupling?

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Excitation contraction coupling (ECC) is a central aspect of cardiac function. ECC consumes a large amount of ATP given by catabolism of fatty acids (FA). Carnitine, a molecule brought mainly by diet, is essential in the FA catabolism. Association of FA with carnitine forms intermediate metabolites, acyl-carnitine (AC), to enter mitochondria where the FA's beta oxidation occurs. Although, disturbances of serum carnitine or AC (increase or decrease) are involved in several cardiomyopathies, the effects of long chain AC, such as palmitoyl-L-carnitine (PC), on different component of ECC: action potential (AP) and intracellular calcium homeostasisare not fully understood. In the present study, AP were recorded on left ventricular papillary muscle of mouse C57BL/6 incubated with different PC concentrations (0, 3 and 10µM) using the microelectrode approach. Application of 10µM PC decreased the AP duration and significantly increased its peak amplitude. In parallel, intracellular calcium homeostasis was analyzed using confocal microscope. Freshly isolated cardiomyocytes were loaded with the calcium dye fluo-4-AM. Under field stimulation; we first measured the dynamic variations of cytosolic calcium (Ca^{2+} transients) at 1Hz. Then, we recorded the activity of ryanodine receptors by measuring spontaneous Ca^{2+} releases from the sarcoplasmic reticulum (Ca^{2+} sparks). We observed a dose-dependent reduction of the SR calcium released associated with some modifications of the kinetics. The rate of release and relaxation were slowed down. At the molecular level, Ca^{2+} sparks frequency dose-dependently increased. These results suggest that AC increases SR Ca^{2+} leak and shortens AP which might serve as is a pro-arrythmogenic substrate in pathophysiological conditions.

0111

The TRPM4 channel in hypoxia-reoxygenation induced mouse ventricular arrhythmias

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Background and purpose: $[Ca^{2+}]_i$-sensitive pathways that are able to prolong the action potential (AP) are key players in early after depolarizations (EADs) induction. Among these, the non-selective cationic channel TRPM4 is a new candidate (Guinamard *et al.* 2011). By producing cell depolarization during rise in calcium, TRPM4 likely participates in cardiac perturbations such as EADs observed during hypoxia and reoxygenation. To investigate this hypothesis, we evaluate the effect of TRPM4 inhibitors on a mouse model of hypoxia-reoxygenation induced arrhythmias.

Experimental approach: Transmembrane potential was recorded using the conventional intracellular microelectrode technique after isolation of right ventricle from mice. The specimen was superfused in standard, normoxic conditions (physiological solution saturated with 95% O_2 and 5% CO_2) during 15min for stabilisation and spontaneous electrical activity was recorded. The preparation was then submitted to hypoxic conditions by turning off the bubbling of the physiological solution for 2 hours. At the end of hypoxic episode, the ventricle was superfused with the initial oxygenated solution, simulating reoxygenation. EADs appeared in all experiments in hypoxic conditions and their occurrence increased after reoxygenation.

Results: Superfusion with the non selective cation channel inhibitor flufenamic acid (10^{-5}mol/L) during the reoxygenation phase significantly reduced the hypoxia-reoxygenation induced arrhythmias (0.3±0.2 compared with 1.9±0.4 EAD/AP in control, n=5). Similary the TRPM4 inhibitor 9-phenanthrol significantly and reversibly decreased the number of EADs. EADs occurrence was reduced by 59.5±12.3% (n=6) and totally abolished at 10^{-4}mol/L (n=6).

Conclusions: TRPM4 pharmacological inhibition leads to a dramatic dose-dependent abolition of hypoxia-reoxygenation induced EADs.

Guinamard R. *et al*, Adv Exp Med Biol. 2011; 704:147-171

0405

Improved survival by cardiac electrophysiologic study-based management of myotonic dystrophy type 1 and overt disease of the conduction system
A propensity analysis

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Context: Up to 1/3 of patients with myotonic dystrophy type 1 (DM1) die suddenly. No intervention has, thus far, effectively prevented sudden death (SD) in DM1.

Objective: To determine whether an invasive strategy (IS) based on systematic electrophysiological study (EPS) and prophylactic permanent pacing prolongs the survival of patients with DM1 and major infranodal conduction delays.

Design: The DM1 Heart Registry prospectively included 914 patients between January 2000 and December 2009.

Setting: Neurological Unit of Pitié-Salpêtrière, a teaching medical center in Paris, France.

Patients: 914 patients with genetically confirmed DM1.

Interventions: Among 486 patients whose electrocardiogram showed a PR interval >200ms, a QRS duration >100ms, or both, we compared the outcome of 341 (70.1%) who underwent IS *versus* 145 (29.8%) who underwent non-invasive strategy (NIS). We used a propensity-score risk adjustment and propensity-based matching analysis to account for selection biases. Main outcome measures: Overall survival (main measure), SD, respiratory death and other deaths (secondary measures).

Results: The median follow-up was 7.4 years (range 0 to 9.9). The 9-year survival rate was higher in the IS (79.0%; 95% CI 73.6-84.8) than in the NIS (69.2%; 95% CI 59.7-80.3) group. Regardless of the statistical technique used to adjust for differences in baseline characteristics between the two groups, IS was associated with a longer survival, with hazard ratios (HR) ranging from 0.53 (95% CI 0.33-0.86, P=.010) to 0.59 (0.37-0.95, P=.031). The survival difference was attributable only to a lower incidence of SD (4.1%; 95% CI 2.0-7.4 vs. 18.0%; 95% CI 10.2-27.4), with HR ranging from 0.21 (95% CI 0.08-0.55) to 0.25 (95% CI 0.11-0.56).

Conclusion: The 9-year survival of patients with DM1 and conduction abnormalities was higher when long-term management was based on an initial invasive than on a non-invasive evaluation.

0031

Alteration in levels of mRNA for mechanosensitive ion channels and in early monophasic action potential duration in the right ventricle of pulmonary hypertensive rats

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The activation of mechanosensitive channels (MSCs) by myocardial stretch can affect electrical activity of the heart and may generate arrhythmias. We have investigated the role of MSCs in a rat model of pulmonary hypertension.

Male Wistar rats (200g) were either given a single injection of saline (CON) or a 60mg/kg monocrotaline (MCT) which induced right heart failure within 3-4 weeks. Animals were killed, hearts isolated and Langendorff perfused at 37°C with physiological solution. The level of mRNA in the left ventricular (LV) and right ventricular (RV) myocardium of CON and MCT hearts was measured by real-time RT-PCR for channels thought to be non-specific cation (TRPC1 and 6) and K^+-specific (TREK-1) MSCs. The RV monophasic action potential duration (MAPD) was measured in hearts stimulated at 5Hz, before and after an increase in RV volume by inflation of an indwelling balloon, to the volume that gave maximum force development (CON 78.3±7.0µl, N=6; MCT 101.3±8.3µl, N=8, $P<0.05$).

In the RV of MCT hearts there was a significant decrease in the mRNA levels for TRPC-1 and TREK-1 but a significant increase in TRPC6 compared to both RV CON and LV MCT (N=10 CON; 12 MCT $P < 0.01$ 2-Way ANOVA). Based on the threshold cycle number TRPC-1 and TREK-1 were more abundant than TRPC-6. The MAPD at 25% repolarisation was significantly reduced by stretch in CON hearts but not MCT hearts ($P < 0.05$). There was no effect on MAPD at 90% repolarisation in either group.

Our observations are consistent with the activation of TRPC and TREK channels in CON hearts, both of which act to shorten the early MAPD, but have opposing effects during the late MAP. In MCT hearts decreased levels of these channels should result in a decreased effect on early MAPD, as we observe. Decreased expression of MSCs in pulmonary hypertensive rats may be a response to the chronic pressure and volume overload that occurs, in order to prevent excessive activation of MSCs.

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